

AMENDMENT OF CLAIMS

TITLE OF INVENTION

An automated method of metabolic stability analysis of library of compounds by isotope dilution mass spectrometry

INVENTORS :

Hoa D. Nguyen, Trinh D. Nguyen, Duc T. Nguyen

Application number : 10/684,249. Art unit 1639.

Date : 01-23-2007



CLAIM LISTING

We claim

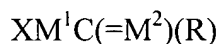
1. (Original) An automated method of determination of metabolic stability of library of compounds from cytochrome P450 enzyme reactions by isotope dilution mass spectrometric analysis comprising the steps of :
 - a) converting a portion of each said library compound into a stable isotope labeled internal standard by addition of a stable isotope labeled chemical reagent; and
 - b) converting another portion of each said library compound into a compound of identical structure, with the exception of the stable isotope atoms, as that of said stable isotope labeled internal standard comprising addition of a non-isotope version of said stable isotope reagent; and
 - c) separating said stable isotope labeled internal standards and said non-isotope labeled converted compounds by an extraction method; and
 - d) incubating other portions of said library of compounds in the presence of cytochrome P450 enzyme systems at body temperature for periods of time; and
 - e) adding quenching chemical reagents to terminate said enzyme reactions; and
 - f) adding said stable isotope labeled internal standards to said terminated reactions; and
 - g) adding said non-isotope version of said stable isotope labeled chemical reagent to said terminated reactions to convert the remaining of each said library compound into said non-isotope labeled converted compounds; and
 - h) separating said converted compound and its internal standard from said reactions by an extraction method; and
 - i) determining the molecular ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and
 - j) determining the most abundant daughter ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and
 - k) determining the ion ratio of said converted compound to said corresponding internal standard for each of said enzyme reactions using tandem mode of mass spectrometric analysis; and
 - l) determining the percent remaining of each said library compound from said enzyme reactions from said ion ratios.
2. (Original) The method of claim 1 wherein said steps a), b), and d) are performed concurrently.
3. (Original) The method of claim 1 wherein said extraction in steps c) and h) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

4. (Original) The method of claim 1 wherein said sample contains either a singularity or a plurality of each class of said library compounds.
5. (Original) The method of claim 1 wherein said library compounds in step a) are converted to said internal standards using a single isotope labeled chemical reagent.
6. (Original) The method of claim 1 wherein said library compounds in steps b) and g) are converted to compounds of identical structure as that of said internal standards, except the labeled atoms, using a single non-labeled version of said chemical reagent.
7. (Original) The method of claim 1 wherein said conversion in steps a), b) and g) are 100% quantitative.
8. (Original) The method of claim 1 wherein the converting step g) is performed before the extraction step h).
9. (Original) The method of claim 1 wherein said quenching chemical reagent is an organic solvent.
10. (Withdrawn-currently amended)
11. (Withdrawn-currently amended)
12. (Withdrawn-currently amended)
13. (Withdrawn-currently amended)
14. (Original) The method of claim 1 wherein said cytochrome P450 enzyme systems are cryopreserved or fresh human and animal hepatocytes, microsomes, S9 fractions or solutions containing individual cytochrome P450 isoenzymes.
15. (Withdrawn-currently amended)
16. (Withdrawn-currently amended)
17. (Withdrawn-currently amended)
18. (Withdrawn-currently amended)
19. (New) The method of claim 1 wherein said library compound has the formula

XMR

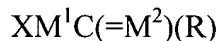
wherein X is H, F, Cl, Br, I, N₃, OR¹, SR¹, CN, NR¹ R², a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R¹ and R² are each independently the same or different linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M is NH, O, CO, and wherein R is H, OH, a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group.

20. (New) The method of claim 1 wherein said converted compound for mass spectrometric analysis has the formula



wherein X is H, F, Cl, Br, I, N₃, OR¹, SR¹, CN, NR¹ R², a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R¹ and R² are each independently the same or different linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M¹ is N, O, CH, CR³, wherein R³ is a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M² is O or NOR⁴, and wherein R⁴ is an alkyl group, and wherein R is H, NR⁵, SR⁵, R⁵, OR⁵, NHR⁵ a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein R⁵ is an alkyl group.

21. (New) The method of claim 1 wherein said stable isotope labeled internal standard for mass spectrometric analysis has the formula



wherein X is H, F, Cl, Br, I, N₃, OR¹, SR¹, CN, NR¹ R², a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R¹ and R² are each independently the same or different linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M¹ is N, O, CH, CR³, wherein R³ is a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group,

and wherein M^2 is O or NOR^4 , and wherein R^4 is a stable isotope labeled alkyl group, and wherein R is H, NR^5 , SR^5 , R^5 , OR^5 , NHR^5 a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein R^5 is a stable isotope labeled alkyl group.

22. (New-Currently amended) The method of claim 1 wherein the stable isotope labeled chemical reagent is selected from a group consisting of a stable isotope labeled acid anhydride, a stable isotope labeled acid chloride, a stable isotope labeled chloroformate, a stable isotope labeled isocyanate, a stable isotope labeled thioisocyanate, a stable isotope labeled alkoxyamine, a stable isotope labeled alkylhydrazine, a stable isotope labeled alcohol and a chloroformate, a stable isotope labeled alkyl halide and a base.
23. (New-Currently amended) The method of claim 1 wherein the non-isotope version of said stable isotope labeled chemical reagent is selected from a group consisting of an acid anhydride, an acid chloride, a chloroformate, an isocyanate, a thioisocyanate, an alkoxyamine, an alkylhydrazine, an alcohol and a chloroformate, an alkyl halide and a base.